IDENTIFICATION OF POLLUTION INDICATOR BACTERIA ISOLATED FROM SOME LAKES, RIVERS, AND PULP AND PAPER WASTEWATER IN SOUTHERN ONTARIO

JUNE 1979

Ontario

Ministry of the Environment

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FINAL REPORT

PROJECT GH 7601

GH 7701

MICRO 7906

IDENTIFICATION OF POLLUTION INDICATOR BACTERIA ISOLATED FROM SOME LAKES, RIVERS, AND PULP AND PAPER WASTEWATER IN SOUTHERN ONTARIO.

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JUNE 1979

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PREFACE

Small freshwater lakes in Ontario were examined from 1970 to 1974 inclusive for the Ontario Ministry of the Environment's Recreational Lakes Program. The objectives for bacteriological studies were to document the present bacteriological water quality of a number of developed recreational lakes and of one undeveloped Lake. This was achieved by determining the density and distribution of fecal bacteria, and the location and sources of major bacterial inputs into these lakes. The program provided much needed information in the field of bacteriological limnology.

Reports on individual lakes were completed by 1975, however, the program was extended to 1977 in a greatly reduced form. The opportunity was then taken to isolate and identify the various groups of pollution indicator bacteria. The principal objective was data quality assurance, but other interesting and important conclusions were drawn from the data.

ACKNOWLEDGEMENTS

This work began in 1976 as an Experience '76 funded project which supported Peter Wood. Wendy Moss was supported by the Lakeshore Capacity Project. Valuable comments, which improved the clarity of the original manuscript, were given by Mr. L. T. Vlassoff and Mr. J. A. Clark, and their assistance is gratefully acknowledged.

SUMMARY

In the first study, total coliforms isolated on m-Endo agar LES from four freshwater lakes in southern Ontario were identified by Enterotube or Minitek rapid identification systems. Coliforms were found to make up about 82% of the isolated sheen colonies. As much as 14% of the sheen colonies were oxidase positive bacteria and therefore non-coliforms. These colonies would contribute to falsely inflated counts. The percentage of the coliforms that were *Escherichia coli*, in the series of lakes, dropped as the trophic status of the lakes increased. This was likely due to the inability of *E. coli* to grow in nutrient rich lakewater while some of the other coliforms can do so.

In the second study, two hundred and forty isolates of fecal coliforms were identified using Enterotube or Minitek rapid identification systems. The fecal coliforms were isolated on MacConkey membrane broth at 44.5^{o}C from a variety of lake, beach and river samples. Fecal coliforms made up 94.5% of the isolates. The majority of the remainder were other Enterobacteriaceae plus a few (2.1%) oxidase positive bacteria which were isolated from the one lake location. The main types of fecal coliforms isolated were *E. coli*, *Klebsiella*, *Enterobacter* and *Citrobacter* with overall frequencies of 70.4%, 15.4%, 5.4% and 3.3% respectively. At polluted sites, a reduced frequency of *E. coli* was observed coupled with greater species diversity among the fecal coliforms. It was suggested that different types of pollution might be recognized through the different genera and proportions of the fecal coliforms.

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The third study illustrated a special case of industrial pollution where wastewater from a pulp and paper mill contaminated the receiving rivers waters. In this study, 191 isolates of fecal coliforms were identified using Enterotube and the Encise III system of identification. The fecal coliforms were isolated from the outfall of the Abitibi forest products mill at Sturgeon Falls and the Sturgeon River above and below the mill. The main types of fecal coliforms isolated were Klebsiella pneumoniae and Escherichia coli with overall frequencies of 49.7% and 33.0% respectively. E. coli was the only fecal coliform isolated above the forest products plant. The types of fecal coliforms isolated from the river below the plant resembled those fecal coliforms from the main plant outfall. The source of K. pneumoniae was clearly the forest products mill. The presence of E. coli in the mill outfall proved that some of the fecal coliforms had a fecal source. The presence of large numbers of K. pneumoniae in the mill wastewater likely introduced an additional public health hazard to users of the river water downstream from the forest products mill.

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Part 1

Total Coliform Isolates From Water of Recreational Lakes in Southern Ontario,

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INTRODUCTION

In 1975, a review was made of the interpretation problems experienced with recreational lakes surveys in previous years. Bacteriological water quality of recreational lakes was measured by three parameters, total coliform, fecal coliform, and fecal streptococcus. Problems arose when one of the parameters diverged greatly from expected levels unaccompanied by similar changes in the other parameters.

Occasionally very high levels of total coliforms were found without high levels of fecal coliforms which suggested that the total coliforms were of a non-fecal type. Two examples were MacLean Lake (14) and Trout Lake (15). The dominant total coliform in Trout Lake was identified as Enterobacter aerogenes, a bacterium more typical of soil and water uncontaminated with fecal material.

An analysis of total coliforms isolated from Lake Ontario on m-Endo broth showed that a large number were not coliforms but belonged to the genus Aeromonas (2). Similar results were obtained for an analysis of total coliforms isolated from Lake Ontario on m-Endo agar LES (1). These reports suggest that levels of bacteria isolated on m-Endo media would not always be a suitable indication of fecal pollution. There have apparently been no previous reports on the identification of total coliforms on m-Endo agar LES from small freshwater recreational lakes.

Two questions can be raised now. What are the principal types of total coliforms isolated from recreational lakes? Does the lack of specificity of the analyses lead to errors in interpretation? The identification of isolates of the bacterial parameters was considered part of a quality assurance program. The first part, concerning the reproducibility of data, was reported recently (9).

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METHODS

Bacterial Enumeration:

Total coliform (TC) bacteria were determined as a count of dark red colonies with gold metallic sheen grown on a membrane filter (MF) (Gelman GN-6) with m-Endo agar LES (7).

Chlorophyll a:

Water samples from mid-lake stations through the photic zone were stabilized with $MgCO_3$ suspension, filtered onto a membrane filter of 1.2 µm pore size, then stored on ice. Chlorophyll a was determined by the MOE Chemistry Section using the method of Richards and Thompson (13). The results are expressed as an arithmetic mean summer Chl a concentration. Sampling for Chl a was carried out by regional technical support personnel.

Treatment of Isolates:

A representative number of sheen colonies was picked from uncrowded plates, streaked on MacConkey agar (Difco) and restreaked on nutrient gelatin plates.

Bacterial Identification:

An oxidase test and Gram stain were carried out on isolated colonies from the nutrient gelatin medium. Biochemical identification tests were carried out using Minitek, (BBL) Becton Dickenson Ltd., or Enterotube, Hoffmann-La Roche, Inc., rapid identification systems.

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RESULTS

Escherichia coli, Enterobacter, Klebsiella and Citrobacter were the main types of total coliforms identified, with an average frequency of 21.7%, 21.7%, 20.2% and 18.8% respectively (Table 1.1). In Mississippi Lake, the most polluted of the four lakes in terms of fecal coliform levels, and the most nutrient enriched lake, the frequency of isolation of E. coli dropped to 6.7%, while the dominant total coliforms were Enterobacter and Citrobacter with a frequency of isolation of 40.4% and 26.7% respectively.

Levels of total coliforms in lakes have been shown to be related to lake trophic status, measured by Chl a (8). From this and other information (4), it was expected that some types of total coliforms would grow in nutrient rich lakewater. Since E. coli is the only coliform not expected to grow in lakewater, one can predict that the E. coli fraction of total coliforms will be reduced as the trophic status of the lake is increased. This sort of relationship was confirmed by the data, and is illustrated in Table 1.2.

Aeromonas, an oxidase positive bacterium, is a major contaminant of total coliform estimations by membrane filtration (1, 2) and most probable number technique (11). The frequency of isolation of oxidase positive bacteria from the total coliform estimation, in the study, was a maximum of 14.8% and averaged 8.7% (Table 1.1).

DISCUSSION

Eighty-two percent of the sheen colonies from recreational lakes samples were coliforms, that is Escherichia, Enterobacter, Citrobacter or Klebsiella. The main group of non-coliforms that were isolated from these samples were the oxidase positive bacteria, such as Aeromonas and other unidentified organisms, possibly pneudomonads. The isolation of non-coliforms on this medium

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TABLE 1.1

TYPES OF BACTERIA ISOLATED AS TOTAL COLIFORMS FROM RECREATIONAL LAKES IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS.

Lake	Citrobacter Species		Enterobacter Species		Escherichia Coli		Klebsiella pneumoniae		Other Klebsiella Species		Serratia Species	
Cloudy, McCarroll Diamond	2/10	20.0%	1/10	10.0%	4/10	40.0%	1/10	10.0%	1/10	10.0%	0/10	0.0%
Kamiskotia	2/17	11.8%	5/17	24.9%	2/17	11.8%	5/17	24.9%	0/17	0.0%	2/17	11.8%
Mississippi	4/15	26.7%	6/15	40.0%	1/15	6.7%	0/15	0.0%	1/15	6.7%	0/15	0.0%
Vermillion	5/27	18.5%	3/27	11.1%	8/27	29.6%	5/27	18.5%	1/27	3.7%	0/27	0.0%
Total Isolates	13/69	18.8%	15/69	21.7%	15/69	21.7%	11/69	15.9%	3/69	4.3%	2/69	2.9%

TABLE 1.1 (Continued)

TYPES OF BACTERIA ISOLATED AS TOTAL COLIFORM FROM RECREATIONAL LAKES IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS.

Lake	Other Bacterial * Species		Oxidase Positive Bacteria						
			Aeromonas Species		Unidentified Oxidase Positive Bacteria		Total Oxidase Positive Bacteria		Toal Isolates
Cloudy, McCarroll Diamond	1/10	10.0%	0/10	0.0%	0/10	0.0%	0/10	0.0%	10
Kamiskotia	0/17	0.0%	1/17	5.9%	0/17	0.0%	1/17	5.9%	17
Mississippi	2/15	13.3%	0/15	0.0%	1/15	6.7%	1/15	6.7%	15
Vermillion	1/27	3.7%	0/27	0.0%	4/27	14.8%	4/27	14.8%	27
Total Isolates	4/69	5.8%	1/69	1.5%	5/69	7.3%	6/69	8.7%	69

* Other Bacterial Species

Alcalescens dispar

Edwardsiella

Proteus morgani

(m-Endo agar LES) presents opportunities for errors in interpretation of coliform data. The magnitude of these errors may be greater than that illustrated by the receational lakes data. The results obtained by other workers were compared to our own in Table 1.3, where it can be seen that, depending on the source of the sample, as much as 43% oxidase positive bacteria can be isolated on m-Endo agar LES.

There is some evidence to suggest that the types of bacteria isolated varied with the sample source. The pattern of results for recreational lakes clearly differed from the results from the Toronto Harbour which was polluted by sewage effluents, urban runoff, and industrial wastes. The difference found was mainly the higher percentage of oxidase positive bacteria from Toronto Harbour samples. A pattern was seen within the recreational lakes data where the fraction of coliforms identified as E. coli decreased as the trophic status of the lakes sampled increased (Table 1.2). Others have reported that the percentage of confirmed coliforms varied with the sample source, being highest in the unpolluted offshore waters of Lake Superior, and lowest in the more polluted waters of Lake Ontario (4).

Clearly total coliform data cannot be interpreted directly to indicate only fecal pollution. There is, however, some merit in considering total coliforms as an indicator of pollution by nutrient materials, which would not be specific for fecal material, but would include it. A good example of this situation is the relationship between trophic status of recreational lakes and the levels of coliforms (8). A total coliform count is useful in other situations where a strictly fecal interpretation is not required. Total coliforms in chlorinated sewage treatment plant effluents can be used to monitor the effectiveness of the chlorination process. Total coliforms play a role in drinking water analysis, when their presence indicates inadequate water treatment (4, 12). In this respect it is useful to remember that 'Standard Methods' (16) retains the total coliform test as the best available indicator of the sanitary pollution of municipal water supplies.

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TABLE 1.2

PERCENTAGE OF TOTAL COLIFORMS THAT WERE E. COLI AS RELATED TO LAKE TROPHIC STATUS.

Lake	Chl a (μg/l)	E. coli % of Total Coliforms
Cloudy, McCarroll & Diamond	* 1.2	40
Vermillion	* 2.65	30
Kamiskotia	* 3.2	12
Mississippi	† 2.0	7

* (Personal communication - G. Myslik, MOE Sudbury)

† (Personal communication - M. German, MOE Kingston)

Mississippi Lake is very productive and the Chl a level is not a good measure of trophic status.

TABLE 1.3

COMPARISON OF TYPES OF COLIFORM (SHEEN) BACTERIA FROM VARIOUS BODIES OF WATER USING m-ENDO MEDIA.

MEDIUM	m-Endo Broth			m-Endo agar LES		
Source of Water	Toronto Harbour (Sheen)[1]	Toronto Harbour (Non-Sheen)[1]	Lake Ontario (Sheen)[1]	Kennebecasis River (Sheen)[2]	Recreational Lakes (Sheen)[3]	Lake Ontario (Sheen)[4]
Escherichia %	13	2	7	20	22	12
Citrobacter %	12	1	7	10	19	4
Enterobacter %	14	5	28	30	22	34
Klebsiella %	15	5	13	20	20	21
Other Enterobacteriaceae %	-	2	-	10	8	-
Oxidase positive (Aeromonas and Pseudomonas) %	43	77	40	10	9	27

1. Taken from Bennett, 1969 (2)
2. Taken from Brown & Tracey, 1974 (3)
3. This report
4. Taken from Bell and Vanderpost, 1973 (1).

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The lack of specificity of total coliform media for their prime target organism, E. coli, has led to some unexpected and interesting bonuses. A great deal of information has been collected incidentally on the distribution of other coliform bacteria. This has led to the conclusions that Klebsiella pneumoniae was the most frequently isolated coliform in paper mill slimes (5), and that in sewage and effluent samples the genus Klebsiella was isolated more frequently than any other coliform on m-Endo agar LES (6). High densities of Klebsiella pneumoniae were also found in a recent MOE survey of industrial pollution (10), and there has been increased interest in further surveillance for this organism in sewage treatment plant effluents.

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Part II

Fecal Coliform Isolates From Water of Recreational Lakes in Southern Ontario

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INTRODUCTION

High levels of fecal coliforms were occasionally found without accompanying levels of fecal streptococci. This suggested bacteria capable of growth on the fecal coliform isolation medium but possibly from a non-fecal source. Under nutrient-rich conditions Klebsiella pneumoniae can grow in water and appear to be from a fecal source. In localized areas this type of problem has been noted in Lake Manitouwabing (14) and Kashwakamak Lake (13).

Very high densities of fecal streptococci were frequently found at inflowing streams where fecal coliforms were absent or at very low levels. Fecal streptococci are more long lived in natural waters than fecal coliforms. Fecal streptococci unaccompanied by fecal coliforms could be derived from a very old source of fecal material in which the fecal coliforms had died out, or from a naturally occurring strain of fecal streptococcus, Streptococcus fecaelis var. liquifaciens, growing in an organic rich location like a marsh. A typical example was the northern inflowing stream to Jerry Lake which drained a marshy area (12). The above situations cannot be interpreted firmly without an identification of the fecal indicator bacteria.

Two questions can be raised now. What are the principal types of fecal coliforms isolated from recreational lakes? Does the lack of specificity of the analyses lead to errors in interpretation? The identification of isolates of the bacterial parameters was considered part of a quality assurance program. The first part, concerning the reproducibility of data, was reported recently (7).

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METHODS

Bacterial Enumeration

Fecal coliform (FC) bacteria were determined as a count of acid producing yellow to yellowish-green colonies grown on a membrane filter with MacConkey broth (Difco). Incubation was for 20 hours at 44.5^{o}C in a plastic cakette in a water bath.

Isolation Procedure

A representative number of fecal coliform colonies was picked from uncrowded plates. One plate was used from each sample and five to ten colonies per plate were picked, streaked on MacConkey agar (Difco); and incubated for 18 hours. An isolated lactose fermenting colony was restreaked on nutrient gelatin medium and incubated at 35^{o}C.

Bacterial Identification

The oxidase test and Gram stain were carried out on isolated colonies from the nutrient gelatin medium. Biochemical identification tests were carried out using the Enterotube rapid identification system, Hoffmann-LaRoche Limited. The Encise III system of identification was used without further supplementary tests. Some isolates were identified by Minitek (BBL). Two hundred and forty isolates were treated in this way.

RESULTS

Escherichia coli was most frequently isolated as a fecal coliform with an average frequency of 77.9%. Other types of bacteria isolated included Klebsiella, Enterobacter, Citrobacter and oxidase positive bacteria with frequencies of 7.0%, 4.7%, 2.3% and 3.5% respective (Table 2. 1). Some (2.3%) of the oxidase positive bacteria were identified as Aeromonas. Thermotolerant Aeromonas growing on a fecal coliform medium has not been documented before. On the average Aeromonas appeared to be a small problem, however, all of the thermotolerant oxidase positive bacteria were isolated from the most polluted lake (Mississippi), which contained the greatest densities of fecal coliforms, and where 18.8% of the organisms isolated from the fecal coliform medium were oxidase positive. Greater species diversity was found among the fecal coliforms from the most polluted lake along with a reduced frequency of E. coli (Table 2.1).

The frequency of isolation of fecal coliform types from beach and lake samples were different. The main types of fecal coliforms isolated from beach samples were E. coli (39.7%), Klebsiella (39.7%), Enterobacter (11.0%), Citrobacter (5.5%) and oxidase positive bacteria (2.7%) (Table 2.2). E. coli was less frequently isolated at polluted beaches.

The samples from lakes and beaches were divided into polluted and unpolluted sources. The types of fecal coliforms isolated from polluted sources were quite different from the fecal coliforms from unpolluted sources. The main difference was observed to be a reduction in the relative frequency of E. coli from polluted beaches and lakes (Table 2.3).

TABLE 2.1

TYPES OF BACTERIA ISOLATED AS FECAL COLIFORMS FROM RECREATIONAL LAKES IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS

SOURCE Lakes	Citrobacter Species		Enterobacter Species		Escherichia coli		Klebsiella pneumoniae		Other Klebsiella Species		Serratia Species	
Kamiskotia	0/30	0.0%	0/30	0.0%	26/30	86.7%	3/30	10.0%	1/30	3.3%	0/30	0.0%
Cloudy, McCarroll, Diamond	0/12	0.0%	1/12	8.3%	9/12	75.0%	1/12	8.3%	0/12	0.0%	1/12	8.3%
Vermillion	0/28	0.0%	1/28	3.6%	24/28	85.7%	1/28	3.6%	0/28	0.0%	0/28	0.0%
Mississippi	2/16	12.5%	2/16	12.5	8/16	50.0%	0/16	0.0%	0/16	0.0%	0/16	0.0%
TOTAL	2/86	2.3%	4/86	4.7%	67/86	77.9%	5/86	5.8%	1/86	1.2%	1/86	1.2%

TABLE 2.1 (continued)

TYPES OF BACTERIA ISOLATED AS FECAL COLIFORMS FROM RECREATIONAL LAKES IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS

SOURCE	Unidentified Oxidase Negative Bacteria		Oxidase Positive Bacteria: Aeromonas Species		Oxidase Positive Bacteria: Positive Oxidase Positive Bacteria		Oxidase Positive Bacteria: Total Oxidase Positive Bacteria		Total Isolates
Kamiskotia	0/30	0.0%	0/30	0.0%	0/30	0.0%	0/30	0.0%	30
Cloudy, McCarroll, Diamond	0/12	0.0%	0/12	0.0%	0/12	0.0%	0/12	0.0%	12
Vermillion	2/28	7.1%	0/28	0.0%	0/28	0.0%	0/28	0.0%	28
Mississippi	1/16	6.3%	2/16	12.5%	1/16	6.3%	3/16	18.8%	16
TOTAL	3/86	3.5%	2/86	2.3%	1/86	1.2%	3/86	3.5%	86

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TABLE 2.2

TYPES OF BACTERIA ISOLATED AS FECAL COLIFORMS FROM BEACHES AND RIVERS IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS

SOURCE	Citrobacter Species		Enterobacter Species		Escherichia coli		Klebsiella pneumoniae		Other Klebsiella Species		Serratia Species	
Beaches (Total)	4/73	5.5%	8/73	11.0%	29/73	39.7%	24/73	32.9%	5/73	6.8%	1/73	1.4%
St. Mary's River	0/6	0.0%	0/6	0.0%	5/6	83.3%	1/6	16.7%	0/6	0.0%	0/6	0.0%
Chippawa Creek	2/4	50.0%	0/4	0.0%	1/4	25.0%	1/4	25.0%	0/4	0.0%	0/4	0.0%
East River	0/71	0.0%	1/71	1.4%	67/71	94.4%	0/71	0.0%	0/71	0.0%	2/71	2.8%
Rivers (Total)	2/81	2.5%	1/81	1.2%	73/81	90.1%	2/81	2.5%	0/81	0.0%	2/81	2.5%
Lakes (Total)	2/86	2.3%	4/86	4.7%	67/86	77.9%	5/86	5.8%	1/86	1.2%	1/86	1.2%
Overall Isolates (Total)	8/240	3.3%	13/240	5.4%	169/752	70.4%	31/240	12.9%	6/240	2.5%	4/240	1.7%

TABLE 2.2 (continued)

TYPES OF BACTERIA ISOLATED AS FECAL COLIFORMS FROM BEACHES AND RIVERS IN 1976 AND IDENTIFIED BY ENTEROTUBE OR MINITEK SYSTEMS

SOURCE	Unidentified Oxidase Negative Bacteria		Oxidase Positive Bacteria: Aeromonas Species		Oxidase Positive Bacteria: Unidentified Oxidase Positive Bacteria		Oxidase Positive Bacteria: Total Oxidase Positive Bacteria		Total Isolates
Beaches (Total)	0/73	0.0%	2/73	2.7%	0/73	0.0%	2/73	2.7%	73
St. Mary's River St. Mary's Sludge	0/6	0.0%	0/6	0.0%	0/6	0.0%	0/6	0.0%	6
Chippawa Creek	0/4	0.0%	0/4	0.0%	0/4	0.0%	0/4	0.0%	4
East River	1/71	1.4%	0/71	0.0%	0/71	0.0%	0/71	0.0%	71
Rivers (Total)	1/81	1.2%	0/81	0.0%	0/81	0.0%	0/81	0.0%	81
Lakes (Total)	3/86	3.5%	2/86	2.3%	1/86	1.2%	3/86	3.5%	86
Overall Isolates (Total)	4/240	1.7%	4/240	1.7%	1/240	0.4%	5/240	2.1%	240

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TABLE 2.3

COMPARISON OF FECAL COLIFORMS FROM POLLUTED AND UNPOLLUTED LAKES AND BEACHES

SOURCE		LAKES		BEACHES	
BODY OF WATER		Mississippi	Kamiskotia, Cloudy, Vermillion	Amelia, Sunset	Chaplain, Kinsman, Harmony, Havilland, Birchhaven, MNR Park
FC/100 ml (GM)*		3 to 71	1 to 3	142 to 611	6 to 62
Relative Status		Polluted	Unpolluted	Polluted	Unpolluted
TOTAL ISOLATES		16	70	50	23
E. coli	%	50.0	84.0	18.0	83.0
Citrobacter	%	12.5	7.0	10.0	4.0
Enterobacter	%	12.5	3.0	14.0	4.0
Klebsiella	%	0.0	9.0	55.0	9.0
Others	%	6.0	3.0	0.0	0.0
TOTAL	100.0	100.0	100.0	100.0	100.0
Citrobacter + *Enterobacter*		25.0	10.0	24.0	8.0

Fecal coliform isolates were also identified from river samples (Table 2.2). Not as many rivers were examined as other types of locations. Most samples were taken from the Big East River above Highway 11. There are very few cottages on the river above this highway and the river is relatively unpolluted. The water is brown from leached organic matter, but the water quality is very appealing. In contrast the Chippawa Creek is quite polluted (5, 9). The relative frequency of E. coli among the fecal coliforms was lower in the polluted river. One of the beaches, Amelia Beach, was polluted by the Chippawa Creek.

DISCUSSION

Fecal coliforms from Mississippi Lake sediment were isolated and identified (6) and compared with the types of fecal coliforms from the water in this study. More E. coli were isolated from the sediment than the water (Table 2.4), but part of this difference may be accounted for by the isolation procedure. Clark and Pagel observed that more Klebsiella were isolated from municipal water supplies by MF procedures than a broth technique (2). It is also possible that better temperature control was achieved for the MPN (sediment) procedure.

About 95% of the bacteria isolated as fecal coliforms from rivers and lakes were found to be coliforms, and 70% were found to be E. coli. In a recent MOE report, total coliforms from lakes were identified (10), of which 82% were coliforms, and 22% were E. coli. The fecal coliform method is therefore much more specific for coliforms and for E. coli and it is for this reason that the fecal coliform parameter is the better indicator of fecal pollution. The current MOE procedure for fecal coliforms provided, in this study, reliable data on fecal pollution indicator levels.

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TABLE 2.4

COMPARISON OF FECAL COLIFORM ISOLATES FROM WATER AND SEDIMENT OF MISSISSIPPI LAKE

BACTERIA SPECIES	WATER * %	SEDIMENT † %
Citrobacter	12.5	NIL
Enterobacter	12.5	NIL
E. coli	50.0	93.0
Klebsiella pneumoniae	NIL	NIL
Other *Klebsiella*	NIL	4.7
Serratia	NIL	NIL
Oxidase positive	18.8	NIL
Others	6.2	2.3

* Bacteria Isolated By MF Procedure.

† Bacteria Isolated By MPN Procedure.

Thermotolerant oxidase positive bacteria were not usually isolated by the fecal coliform method. These bacteria were isolated more frequently on total coliform media but may cause occasional problems with the MMB fecal coliform medium.

The most interesting conclusion reached in this study was that there were changes in the frequencies of types of fecal coliforms with increased levels of pollution. At polluted sites there was a reduced frequency of E. coli coupled with greater species diversity among the other fecal coliform types (Table 2.3).

About 95% of the coliforms in feces are E. coli (11). Dufour has shown recently that after fecal material travels through a sewer to reach a sewage treatment plant,.sufficient time has elapsed for considerable changes to have taken place; E. coli drops to about 22%, and the balance is made up of Klebsiella, Citrobacter, and Enterobacter (3). Dufour concluded that such changes made the fecal coliform test less valid and therefore only E. coli should be the measure of fecal pollution. One can conclude in addition that sewage pollution is characterized by the presence of E. coli plus the other coliforms whereas fresh fecal pollution is characterized by E. coli alone or at least at the 95% level.

The pollution in the Big East River was considered to be of animal origin flushed in by rainfall, and about 94% of the fecal coliforms were E. coli (Table 2.2). Whereas fecal pollution in the Chippawa Creek - Amelia Beach area was of human origin and E. coli plus the other coliforms were present. The changes which take place in the composition of fecal coliforms in feces take place when the fecal material is held for some period of time under conditions of high nutrients which allow growth of some of the fecal coliforms. Our data indicates that these conditions may be found in some eutrophic lakes. A variety of fecal coliforms was found in Mississippi Lake which had been polluted by cattle feces and leakage from unsatisfactory septic tanks.

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The generic distribution of the fecal coliforms may give some information on the source of pollution. This approach may be useful when examining industrial wastes. This work is still very much at the speculative stage, but some information is available on the identification of the variety of fecal coliforms from a few industrial sources (15) and in particular pulp and paper mill wastewater, by the MOE (8), and by other investigators (1, 4, 15).

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Part 3

Fecal Coliform Isolates From Pulp and Paper Wastes and Their Receiving Waters:

Sturgeon Falls 1977

LIST OF TABLES

INTRODUCTION

A number of studies (1, 2), conducted by the National Council of the Paper Industry (NCASI), have shown that coliforms in paper mill wastes need not be of sanitary origin and so have made it difficult to apply fecal coliform standards to the pulp and paper waste effluents. The NCASI reports recommended identification of the fecal coliforms isolated, and the authors agreed that E. coli would be a suitable fecal indicator for the paper industry (1). Klebsiella pneumoniae is the principal coliform found in paper mill slimes (1, 2). The known public health danger of these organisms stems from the fact that they appear to be as pathogenic as those isolated from classical human infections. The soundest approach to this problem is to carry out the appropriate epidemiological studies of Klebsiella pneumoniae from the water environment (3). Other pathogens, including Salmonella and Pseudomonas aeruginosa have been isolated from pulp mill wastewater (4, 5).

Following complaints of recreational water quality degradation below Sturgeon Falls, it was decided to survey for inputs of fecal bacteria and perform biochemical tests to identify a representative sample of the fecal coliforms. Pseudomonas aeruginosa and thermotolerant Klebsiella pneumoniae were also determined as they were considered the most numerous pathogenic bacteria likely to be found in the pulp mill wastewater. The results of the water quality survey have been reported (6). This report provides more detailed information on the identification of the fecal coliforms.

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METHODS

Bacterial Enumeration

Fecal coliform (FC) bacteria were determined as a count of acid producing yellow to yellowish-green colonies grown on a membrane filter with MacConkey broth. Incubation was for 20 hrs. at 44.5°C in a plastic cakette in a water bath.

Isolation Procedure

All target colonies, from five to fifteen, were taken from each uncrowded membrane or from a definite sector of a membrane over a three-day period. Samples taken within the mill were taken on one day only. In this way a representative number of fecal coliform colonies were picked, streaked on MacConkey agar, and incubated at 35°C for 18 hours. An isolated lactose fermenting colony was restreaked on nutrient gelatin medium and incubated at 35°C.

Gas production of 133 of the isolates was determined in EC broth at 35°C and 44.5°C after 24 hr. incubation.

Bacterial Identification

The oxidase test and Gram stain were carried out on isolated colonies from the nutrient gelatin medium. In 1977, biochemical identification tests were performed with Enterotubes, Hoffmann-La Roche Ltd. The Encise III system of identification was used without further supplementary tests. One hundred and ninety-one isolates were treated in this way.

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RESULTS

The main types of fecal coliforms isolated were Klebsiella pneumoniae, and Escherichia coli, with overall frequencies of 49.7% and 33.0% respectively (Table 3.1). Oxidase positive bacteria, which by definition are not fecal coliforms, were found at a frequency of 9.9%. A few Enterobacter were isolated with a frequency of 3.7% for E. cloacae, and 0.5% for E. agglomerans. Six of 191 isolates could not be identified using Enterotube. These bacteria gave very few positive biochemical reactions.

Gas was produced by 103 of 133 (77.5%) isolates tested at 44.5°C in EC broth, while 12 isolates (9.0%) produced gas only at 35°C, and 18 isolates (13.5%) did not produce gas under the test conditions (Table 3.2). Only 8 of the identified isolates produced gas only at 35°C, they are therefore categorized as "anaerogenic fecal coliforms". Most of the non-coliforms (non gas producers) were oxidase positive bacteria.

DISCUSSION

If an MPN fecal coliform procedure had been used instead of the present MF method, then 30 of 133 isolates (22.5%) which did not produce gas at 44.5°C, and are therefore not fecal coliforms, would not have been isolated. In this respect, the MPN procedure would be the more accurate one. However, the 8 "anaerogenic fecal coliforms" would have been excluded and they may be of the same origin as the aerogenic or 'true' fecal coliforms. The method used for this survey, that of isolation on a membrane filter followed by identification of a representative sample of bacteria, seems to be the best so far available.

TABLE 3.1

IDENTIFICATION OF FECAL COLIFORMS FROM THE ABITIBI FOREST PRODUCT PLANT AND THE STURGEON RIVER

LOCATION	Fecal Coliform Density per 100 ml	Number of Colonies Identified	Identification	%
Upstream (above dam)	4	14/14	E. coli	100.0
Abitibi (inside Plant) Process sewers	1,500	14/25	K. pneumoniae	56.0
		7/25	E. coli	28.0
		3/25	E. cloacae	12.0
		1/25	Oxidase positive	4.0
Abitibi (inside Plant) Pulp Mill Sewer	266	20/20	E. coli	100.0
Abitibi Outfall	4,900	16/27	K. pneumoniae	59.2
		9/27	Oxidase positive	33.3
		1/27	E. agglomerans	3.7
		1/27	E. coli	3.7
Downstream (below dam) (Aug. 10 - 14)	112	29/53	K. pneumoniae	54.7
		9/53	Oxidase positive	16.9
		7/53	E. coli	13.2
		6/53	No Identification	11.3
		2/53	E. cloacoae	3.8
Downstrem (below dam) June 29	294	36/52	K. pneumoniae	69.2
		14/52	E. coli	26.9
		2/52	E. cloacae	3.9
		0/52	Oxidase positive	nil
Overall		95/191	K. pneumoniae	49.7
		63/191	E. coli	33.0
		19/191	Oxidase positive	9.9
		7/191	E. cloacae	3.7
		6/191	No Identification	3.2
		1/191	E. agglomerans	0.5

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TABLE 3.2

Production of Gas at $35^{o}C$ and $44.5^{o}C$ by Fecal Coliforms Isolated From Pulp and Paper Wastes and their Receiving Waters.

Gas at $44.5^{o}C$		Gas at $35^{o}C$ (Not at $44.5^{o}C$)		No Gas (Either $35^{o}C$ or $44.5^{o}C$)	
Identity of Coliform	Number Colonies	Identity of Coliform	Number Colonies	Identity of Bacteria	Number Colonies
K. pneumoniae	52	*K. pneumoniae*	5	Oxidase positive	15
E. coli	49	*E. cloacae*	2	No identification	3
No identification	2	*E. agglomerans*	1		
		No identification	2		
		Oxidase positive	2		
TOTAL	103	TOTAL	12	TOTAL	18
%	77.5	%	9.0	%	13.5

GRAND TOTAL = 133 isolates.

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A comparison was made between the different types of fecal coliforms isolated at different locations inside the wood processing plant and in the river (Table 3.1). E. coli was the only fecal coliform isolated above the wood processing plant. The fecal coliforms isolated below the plant showed greater species diversities which resulted in the isolation of large numbers of thermotolerant K. pneumoniae and oxidase positive bacteria not isolated above the plant. The types of fecal coliforms isolated from the wood processing plant outfall were similar to those obtained from the downstream samples and dissimilar to those found in samples upstream from the plant. It was concluded that the wood processing plant was the source of the large numbers of thermotolerant K. pneumoniae, oxidase positive bacteria, and Enterobacter enumerated as fecal coliforms in the downstream samples.

Within the plant, the fecal coliforms isolated from the Pulp Mill sewer were different from those isolated from the Process sewers (Harboard Mill and Corrugating Mill sewers) - Table 3.1). The temperature of pulping was greater than that of the other processes and this was likely the cause of the lower densitites of bacteria in the Pulp Mill sewer. Higher temperatures would also select for the most thermotolerant fecal coliform (E. coli) which may also explain why only E. coli was found in the Pulp Mill sewer.

Thermotolerant Klebsiella and Enterobacter have been isolated before from pulp mill wastes and their receiving waters (1, 3, 4 and 5). This report confirmed those findings and also indicated that thermotolerant oxidase positive bacteria can be isolated from pulp mill wastes which further interfered with the interpretation of the fecal coliform values. For example, it has been claimed that fecal coliforms in pulp and paper wastes cannot be used as indicators of fecal contamination without further identification of the fecal bacteria (1). Interpretation is made, in this report, only of the densities of identified species of fecal coliforms.

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E. coli was obtained at low frequency from the fecal coliforms isolated from the plant main outfall and from the sewers within the plant (Table 3.1). *E. coli* represents fecal pollution from some source, and if some of the *E. coli* resulted from growth in the nutrient-rich process waters, then pathogenic bacteria may also have an opportunity to grow. In addition, the Abitibi effluent may pose a possible public health hazard by the presence of large numbers of *K. pneumoniae* in the pulp and paper wastes. The magnitude of this public health hazard will not be known unless suitable epidemiological studies are performed.

This study was included here as a special case of industrial pollution of river water by fecal coliforms. These indicator bacteria need not be from a fecal source, however, and additional tests must be carried out to prove their origin.

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OVERALL CONCLUSIONS

The m-ENDO agar LES medium for total coliforms gave reasonably accurate results on lake samples. False positive colonies can be produced by oxidase positive bacteria. This was documented by our results and a review of the literature was also done to show that poorer results have been found by others using other types of samples with this total coliform medium. The ecological conclusions reached in this study concerning the variation in frequency of types of total coliforms with nutrient level of lakes was based on only a few isolates in each case. The conclusions were interesting but tentative and should be verified with further sampling.

MacConkey membrane broth (MMB) for fecal coliforms gave reasonably specific results on lake and river samples. False positive results were normally few in number. A recent MOE study showed that the performance of MMB can be improved on, and the MOE has accepted a new fecal coliform medium (mTEC) suggested from a series of comparative tests of media (see Pagel, J.E. and L.T. Vlassoff in the proceedings of the 1979 ASM annual conference).

An important conclusion was noted in the pulp and paper waste study. Fecal coliform values obtained by MMB or m-TEC should not be interpreted directly as indicators of fecal pollution because of the presence of large numbers of thermotolerant K. pneumoniae from non-fecal sources. This however is a very special case.

The frequency of types of fecal coliforms varied with the sample source (Table 2.3). It was suggested that this fact could be used to characterize some pollution sources, particularly some industrial wastes. Table 3.3 shows the variation to be expected in rivers polluted with either wild animal, human or pulp and paper wastes.

TABLE 3.3

Variation in frequency of fecal coliform isolates from rivers polluted with animal, human, or industrial wastes.

	Percent Fecal Coliform Type		
Body of Water	East River	Chippawa Creek	Sturgeon River
Pollution Source	Wild Animal	Human *	Pulp and Paper
E. coli	94.4	25.0	20.0
K. pneumoniae	0.0	25.0	61.9
Enterobacter	1.4	0.0	3.8
Citrobacter	0.0	50.0	0.0
Serratia	2.8	0.0	0.0
Unidentified Oxidase negative	1.4	0.0	5.7
Unidentified Oxidase positive	0.0	0.0	8.5

* All of the pollution was not of human origin since the creek also contained runoff water.

The river polluted by wild animals in a natural setting contained a high frequency of E. coli and a low frequency of other types of coliforms, whereas human activity in the Chippawa Creek provided a low level of E. coli with a high frequency of other coliforms. Pulp and paper wastes were characterized by low frequency of E. coli and a high frequency of K. pneumoniae plus the presence of unidentified oxidase negative and oxidase positive bacteria. These conclusions should be verified with further sampling and the range of wastewaters extended in future studies.

	DATE DUE		